SUPPORTING INFORMATION

Mitragynine/corynantheidine pseudoindoxyls as opioid analgesics with mu agonism and delta antagonism, which do not recruit β-arrestin-2

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ADDITIONAL EXPERIMENTAL DATA

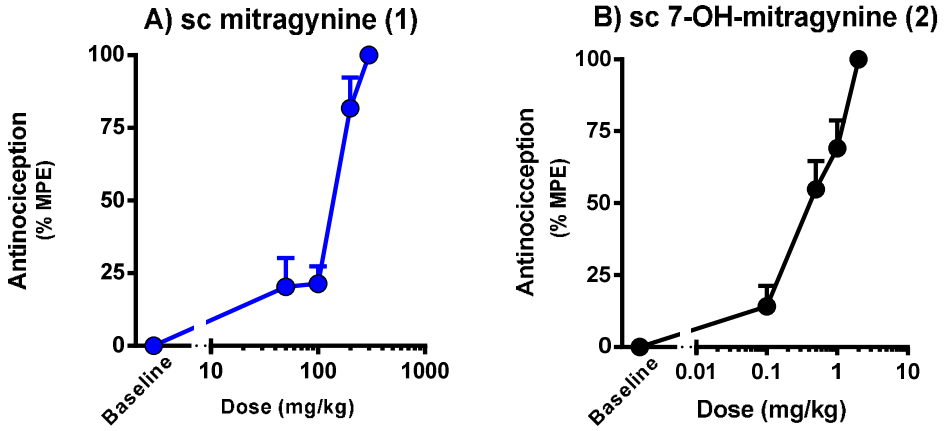


Figure S1. Dose-response curves of antinociception of A) mitragynine (**1**) and B) 7-OH mitragynine (**2**) in CD1 mice. Three independent determinations of the cumulative dose–response curves were performed on groups of mice (n = 10) for antinociception in the tail flick assay. The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100. Points represent mean ± SEM for 30 mice. ED_{50} values (and 95% confidence limits) were: Compound **1**: ED_{50} (CI) = 166 mg/kg (101, 283); compound **2**: ED_{50} (CI) = 0.46 mg/kg (0.39, 0.71).

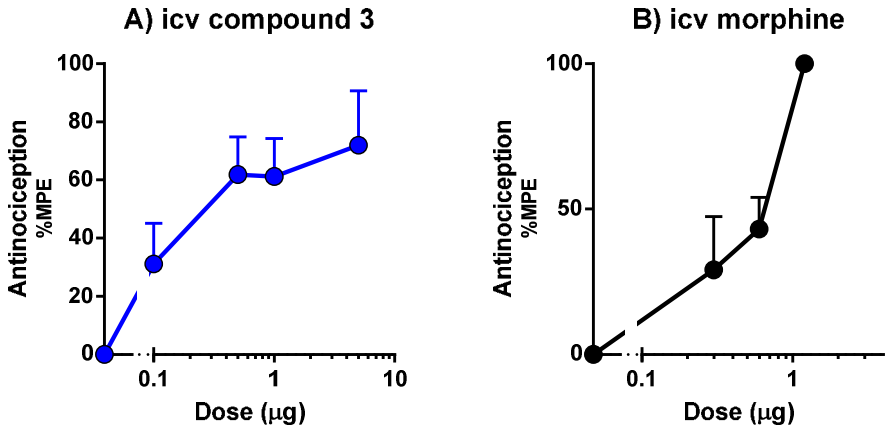


Figure S2. A) Dose-response curves of antinociception of A) **3** and B) morphine given supraspinally in CD1 mice. Two independent determinations of the cumulative dose–response curves were performed on groups of mice (n = 5) for antinociception in the tail flick assay with the compounds given intracerebroventricularly. Animals were tested 15 min later at peak effect to generate the analgesic dose-response curve. Each point represents mean ± SEM for 10 mice. ED_{50} values (and 95% CI) were: compound **3**: 0.38 μg (0.18, 0.81); morphine: 0.53 μg (0.27, 1.0).

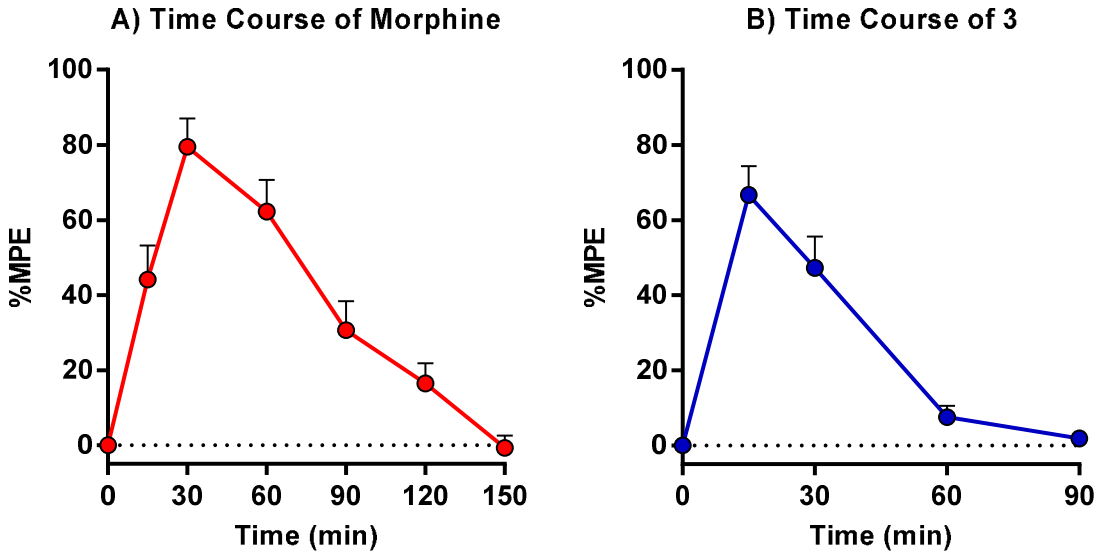


Figure S3. Time course of the antinociceptive effect of A) morphine (4.5 mg/kg) and B) compound **3** (1.5 mg/kg) following sc administration. Two independent determinations were performed on groups of mice (n = 10) for antinociception in the tail flick assay. The means in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100. The area under the curve, an indication of drug exposure, was 2320 for **3** and 5722 for morphine.

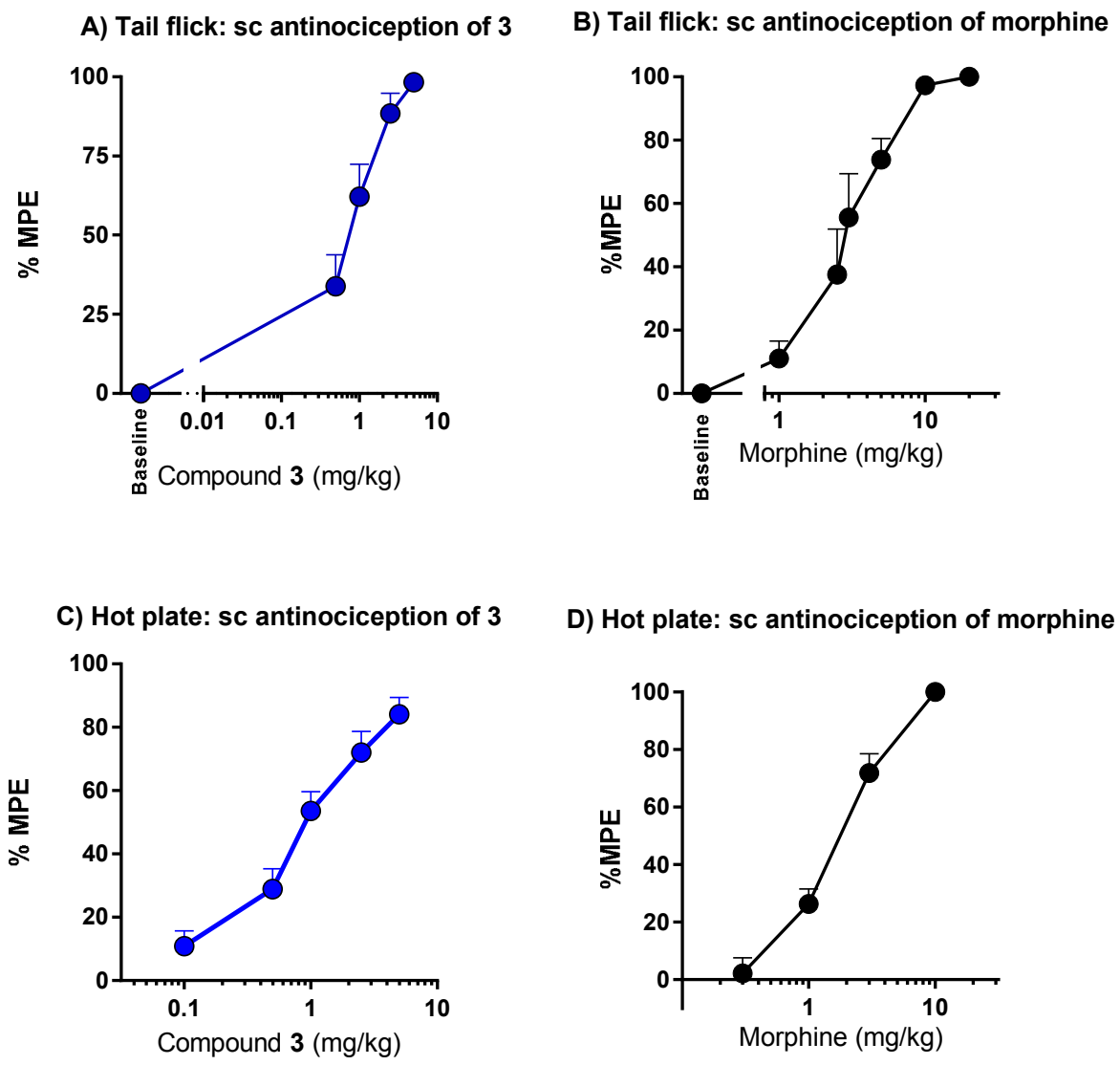


Figure S4. A and B): Tail-flick. Dose-response curves of antinociception of **3** (A) and morphine (B) in CD1 mice. Two independent determinations of the cumulative dose–response curves were performed on groups of mice (n = 10) for antinociception in the tail flick assay. Animals were tested 15 min later (A) and 30 min (B) at peak effect to generate the antinociceptive dose-response curve. Each point represents mean ± SEM for 20 mice. ED_{50} values (and 95% confidence limits) were: compound **3**: ED_{50} (CI) = 0.76 mg/kg (0.56, 0.83); morphine: ED_{50} (CI) = 2.5 mg/kg (1.8, 3.4). C) and D): Hot plate. Groups of CD1 mice (n = 10) were assessed for antinociception of **3** (C) and morphine (D) at peak effect in two independent experiments (n = 20 total) in a cumulative dose-response paradigm. Analgesia was determined using a 55 °C hot plate where the latency to respond with a hind paw lick or shake/flutter, whichever came first, was recorded. ED_{50} values (and 95% confidence limits) were: compound **3**: ED_{50} (95% CI) = 0.99 mg/kg (0.75–1.3); morphine: ED_{50} (CI) = 1.7 mg/kg (1.3, 2.4). The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100.

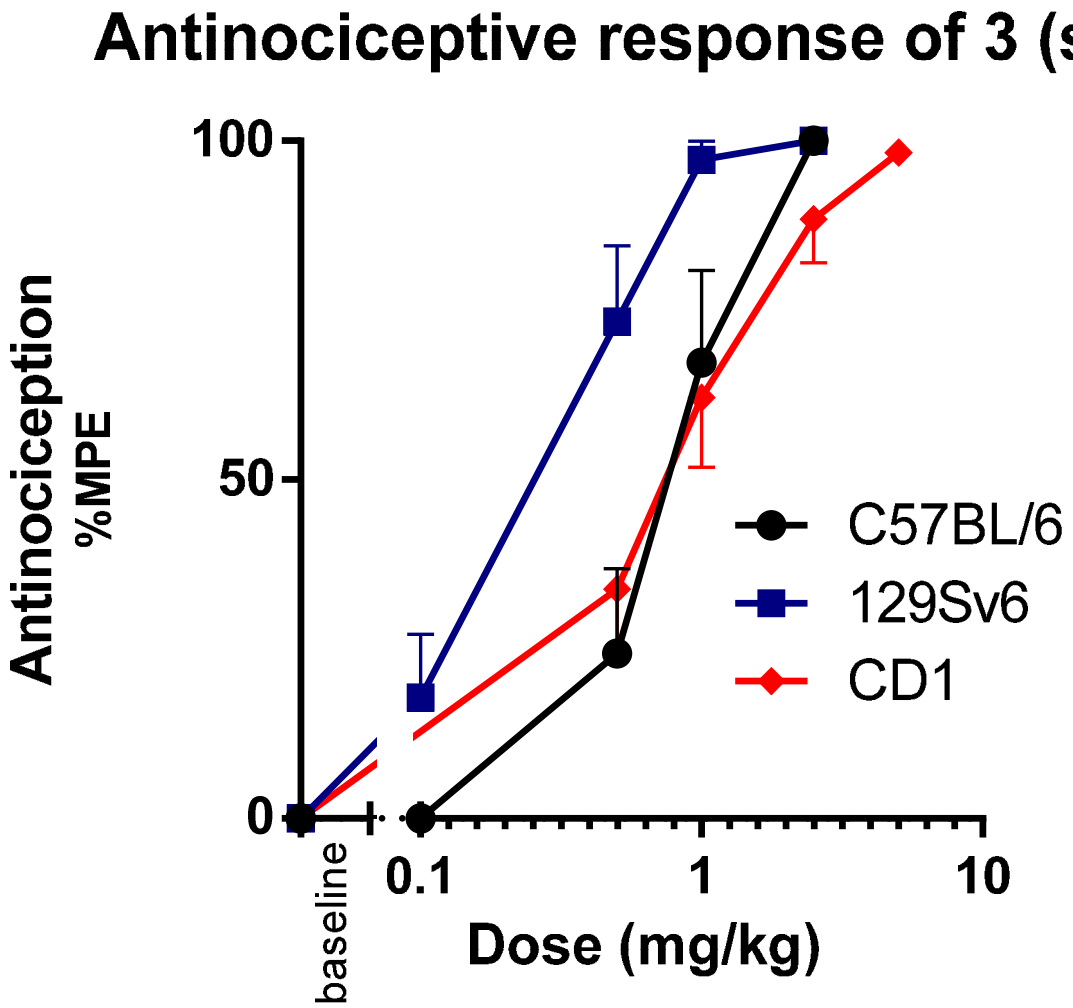


Figure S5. Dose-response curves of antinociception of **3** in C57BL/6, 129Sv6, and CD1 mice. Two independent determinations of the cumulative dose–response curves were performed on groups of mice (n = 10) for antinociception in the tail flick assay. Animals were tested 15 min later at peak effect to generate the antinociceptive dose-response curve. The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100. Each point represents mean ± SEM for 20 mice. ED_{50} values (and 95% confidence limits) were: C57: ED_{50} (CI) = 0.76 mg/kg (0.59, 0.98); 129Sv6: ED_{50} (CI) = 0.25 mg/kg (0.18, 0.36); CD1: ED_{50} (CI) = 0.76 mg/kg (0.56, 0.83).

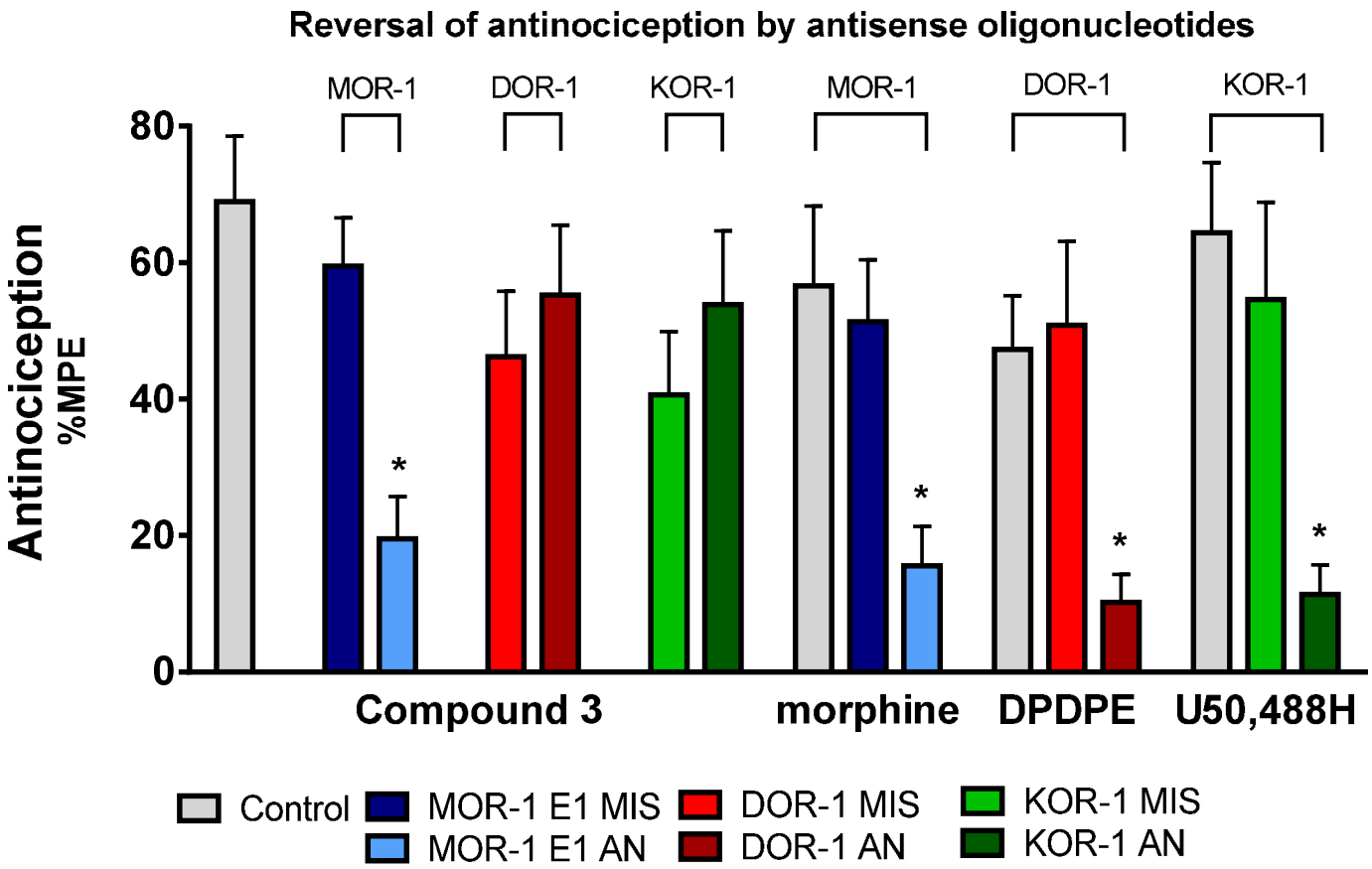


Figure S6. Antisense oligodeoxynucleotide injection: Groups of mice received the stated antisense (5-10 µg) or mismatch (5-10 µg) oligodeoxynucleotide icv under light isoflurane anesthesia on days 1, 3 and 5. Tail flick antinociception was tested on day 6. Control groups received no injection prior to testing. On test day, mice received **3** (1.5 mg/kg, sc), morphine (0.75 µg, icv), DPDPE (10 µg, icv), or U50,488H (5 mg/kg, sc). All experiments were performed 3 times with similar results observed with each determination. *Significantly different from the control within each group ($p < 0.05$). Sequences of AN and MIS oligos are shown in table 4 (experimental section).

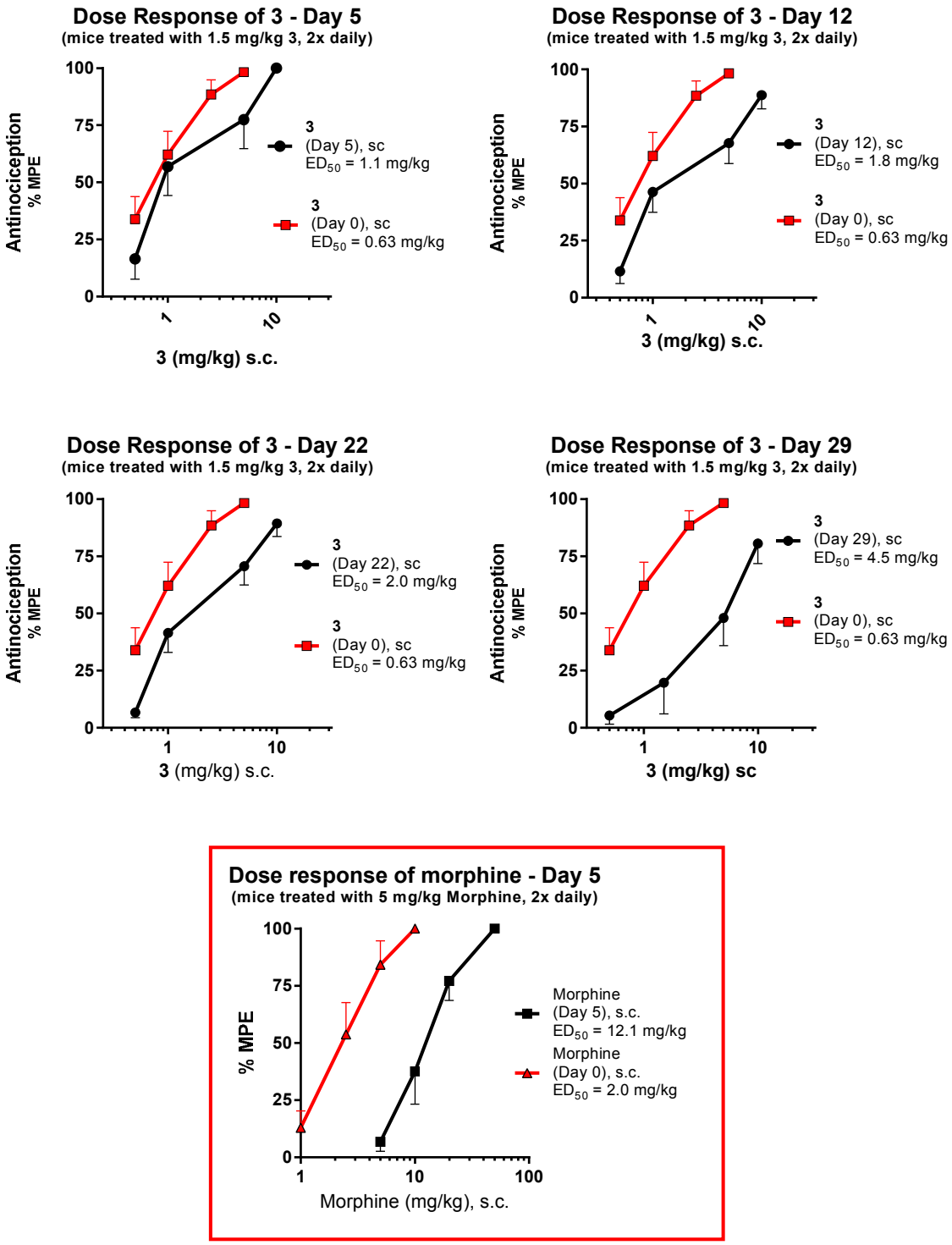
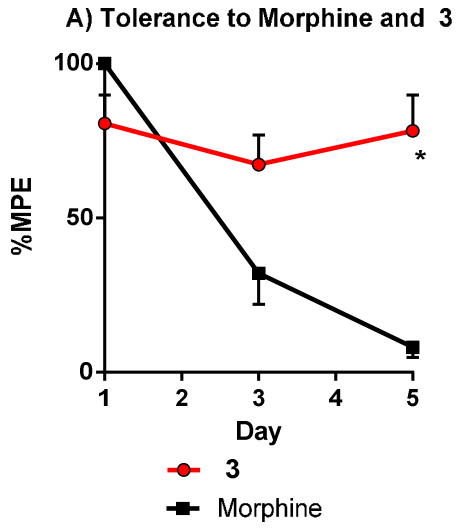
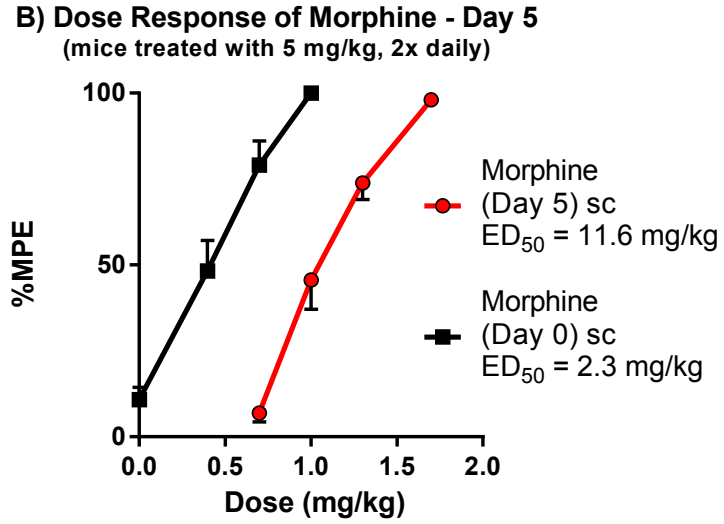


Figure S7. Development of tolerance to **3** and morphine. Groups of CD1 male mice (n=10) were treated with either morphine (2 x ED_{50}, 5 mg/kg) or **3** (2 x ED_{50}, 1.5 mg/kg) twice per day subcutaneously at dosing intervals of 12h. Determinations of the cumulative dose–response curves were performed on the morphine group and **3** on day 5. Separate groups of CD1 mice (n=10 per group) were treated with **3** for

12, 22, and 29 days. Cumulative dose–response experiments were performed with **3** on the respective group on days 12, 22, and 29. The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100. Each point represents mean ± SEM for 10 mice. ED_{50} values (and 95% confidence limits) were: Morphine, naïve (day 0): 2.0 mg/kg (1.2, 3.3); day 5: 12.1 mg/kg (7.6, 19.4). Compound **3**: naïve (day 0): 0.63 mg/kg (0.42, 0.95); day 5: 1.1 mg/kg (0.66, 2.0); day 12: 1.8 mg/kg (1.2, 2.7); day 22: 2.0 mg/kg (1.4, 2.8); day 29: 4.5 mg/kg (2.7, 7.7).


A) Tolerance to Morphine and 3
100
50
0
%MPE
*
1
2
3
4
5
Day
3
Morphine


B) Dose Response of Morphine - Day 5
(mice treated with 5 mg/kg, 2x daily)
100
50
0
%MPE
0.0
0.5
1.0
1.5
2.0
Dose (mg/kg)
Morphine
(Day 5) sc
ED50 = 11.6 mg/kg
Morphine
(Day 0) sc
ED50 = 2.3 mg/kg

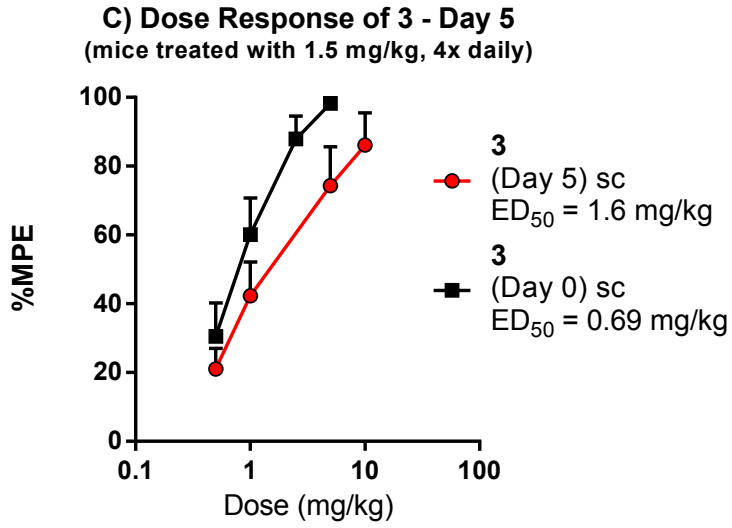

C) Dose Response of 3 - Day 5
(mice treated with 1.5 mg/kg, 4x daily)
100
80
60
40
20
0
%MPE
0.1
1
10
100
Dose (mg/kg)
3
(Day 5) sc
ED50 = 1.6 mg/kg
3
(Day 0) sc
ED50 = 0.69 mg/kg

Figure S8. Development of tolerance to **3** and morphine. Groups of CD1 male mice (n=10) were treated with either morphine (2 x ED_{50}, 5 mg/kg, twice per day) or **3** (2 x ED_{50}, 1.5 mg/kg, four times per day) subcutaneously. A) Antinociceptive tolerance: **3** showed very little tolerance compared with morphine on Day 5 even under a 4 times per day dosing paradigm. On day 5, the antinociceptive effect of **3** was significantly greater than that of morphine (t-test, $P < 0.0001$). Determinations of the cumulative dose–response curves were performed on the morphine group (B) and **3** (C) on day 0 and 5. The means of each dose in each determination were determined as percentage maximal possible effect (%MPE) [(observed latency − baseline latency)/(maximal latency − baseline latency)] x 100. Each point represents mean ± SEM for 10 mice. ED_{50} values (and 95% confidence limits) were: Morphine, naïve (day 0): 2.3 mg/kg (1.7, 3.1); day 5: 11.6 mg/kg (8.7, 15.4). Compound **3**: naïve (day 0): 0.69 mg/kg (0.46, 1.0); day 5: 1.6 mg/kg (0.97, 2.6).

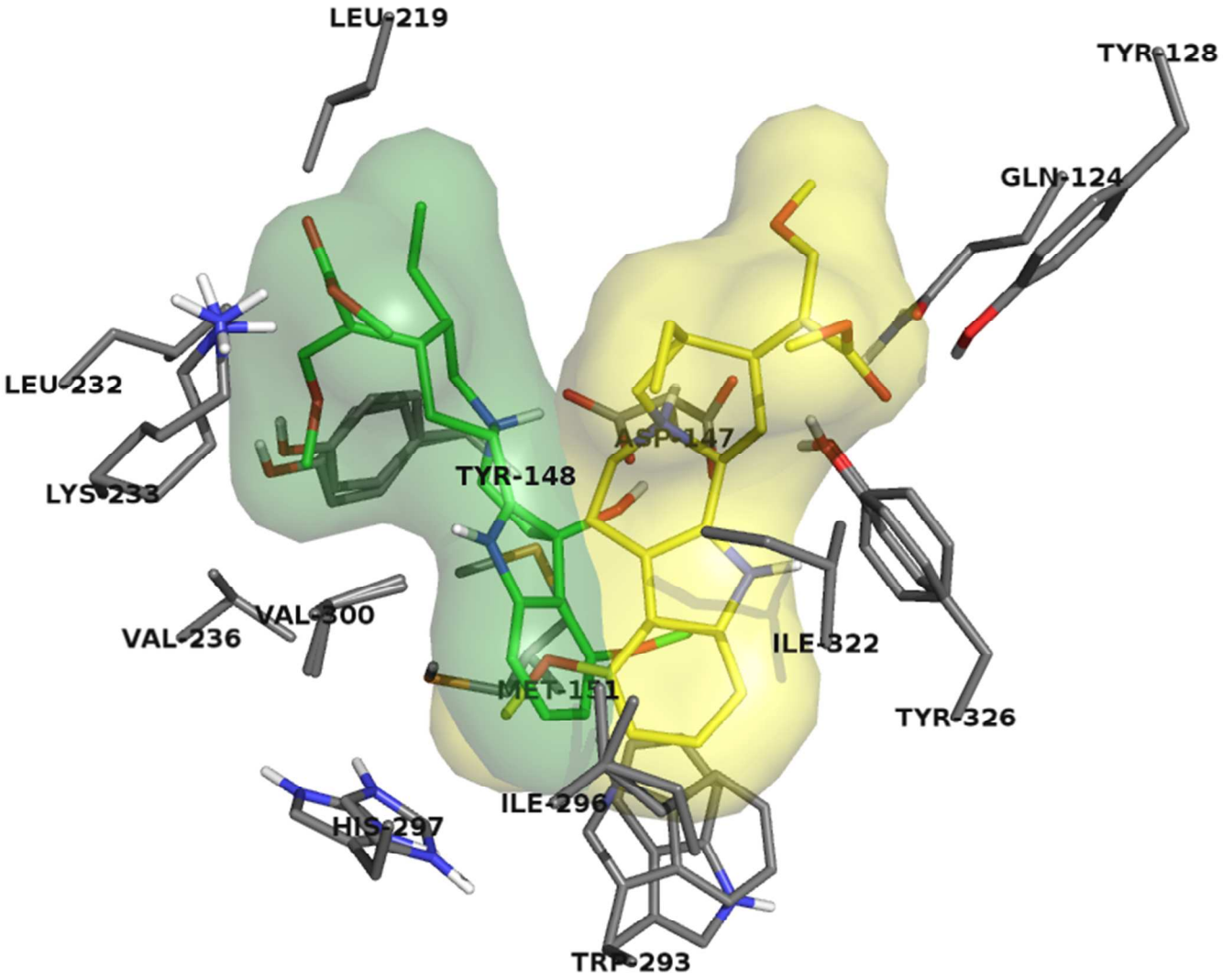


Figure S9. Differences in the binding orientation of **1** (yellow) and **2** (green) bound to mu receptor.

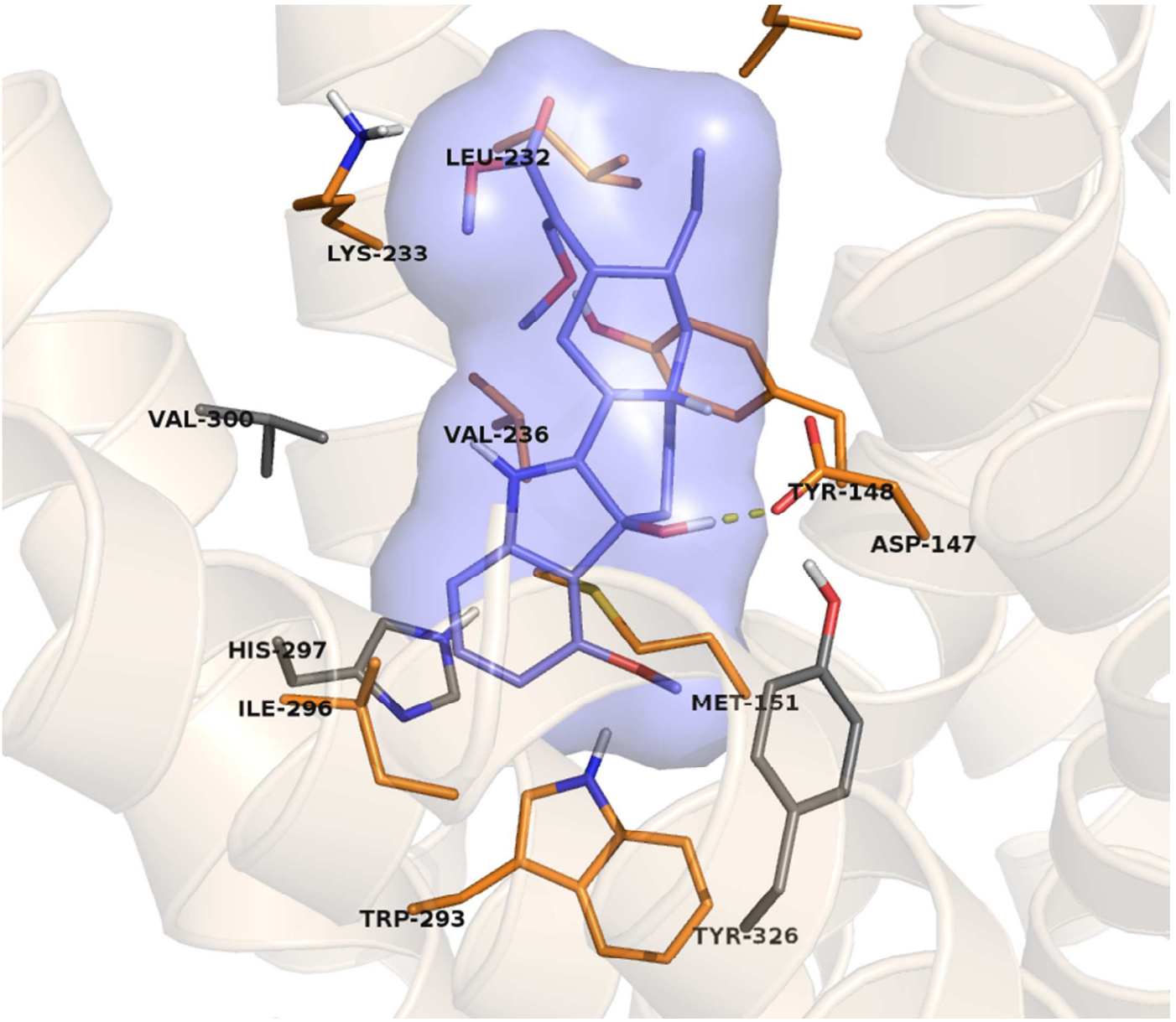


Figure S10. Compound **2** docked to mu receptor. The yellow dotted line highlights a hydrogen bonding interaction between the C-7 hydroxyl group and Asp[147].

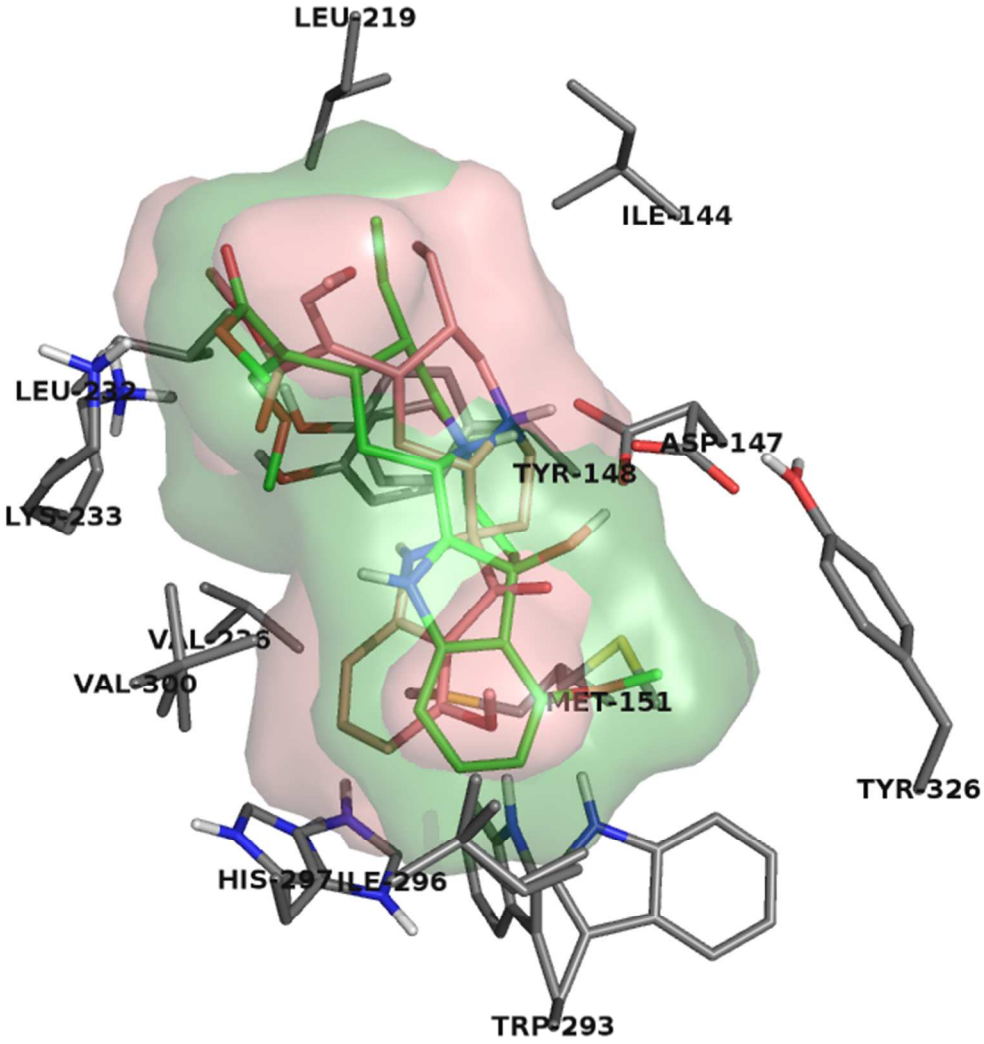


Figure S11. Differences in the binding orientation of **2** (green) and **3** (salmon) bound to mu receptor.

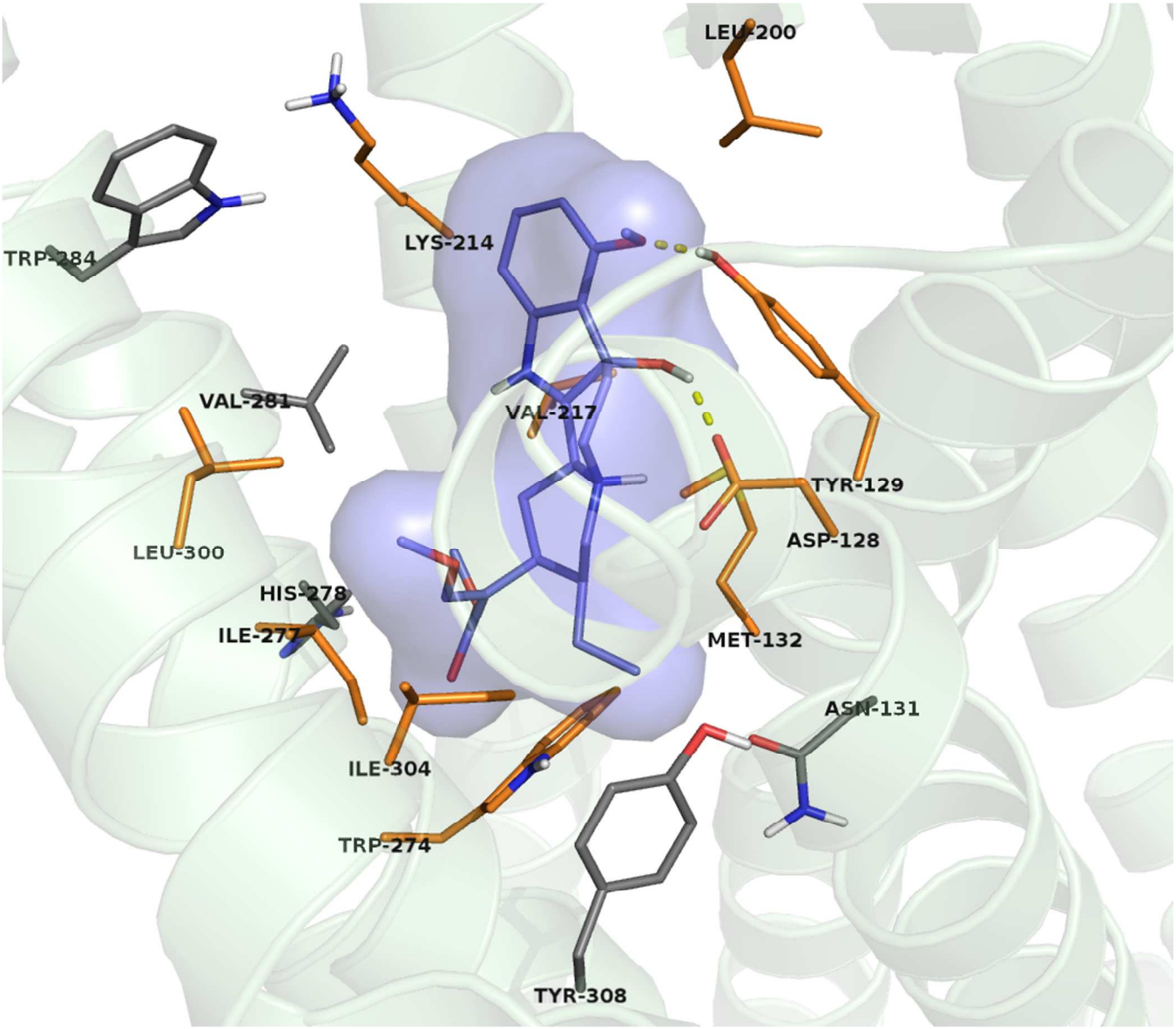


Figure S12. Compound **2** docked to delta receptor. The yellow dotted lines highlight hydrogen bonding interactions between the C-7 hydroxyl group and Asp^{128} and between the C-9 methoxy and Tyr^{129}.

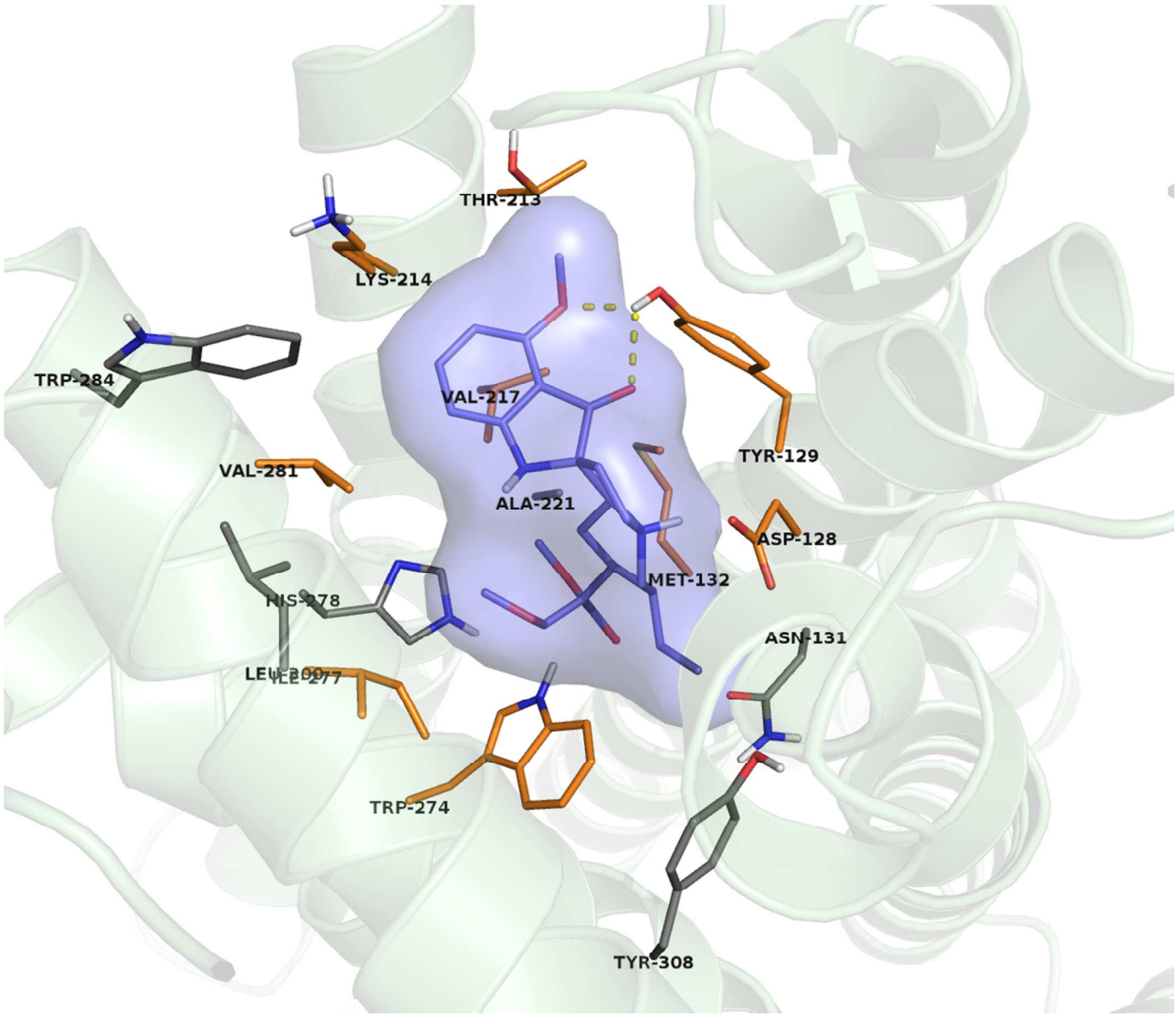


Figure S13. Compound **3** docked to delta receptor. The yellow dotted line highlights a hydrogen bonding interaction between Asp[128] and the C-7 oxo and C-9 methoxy groups.

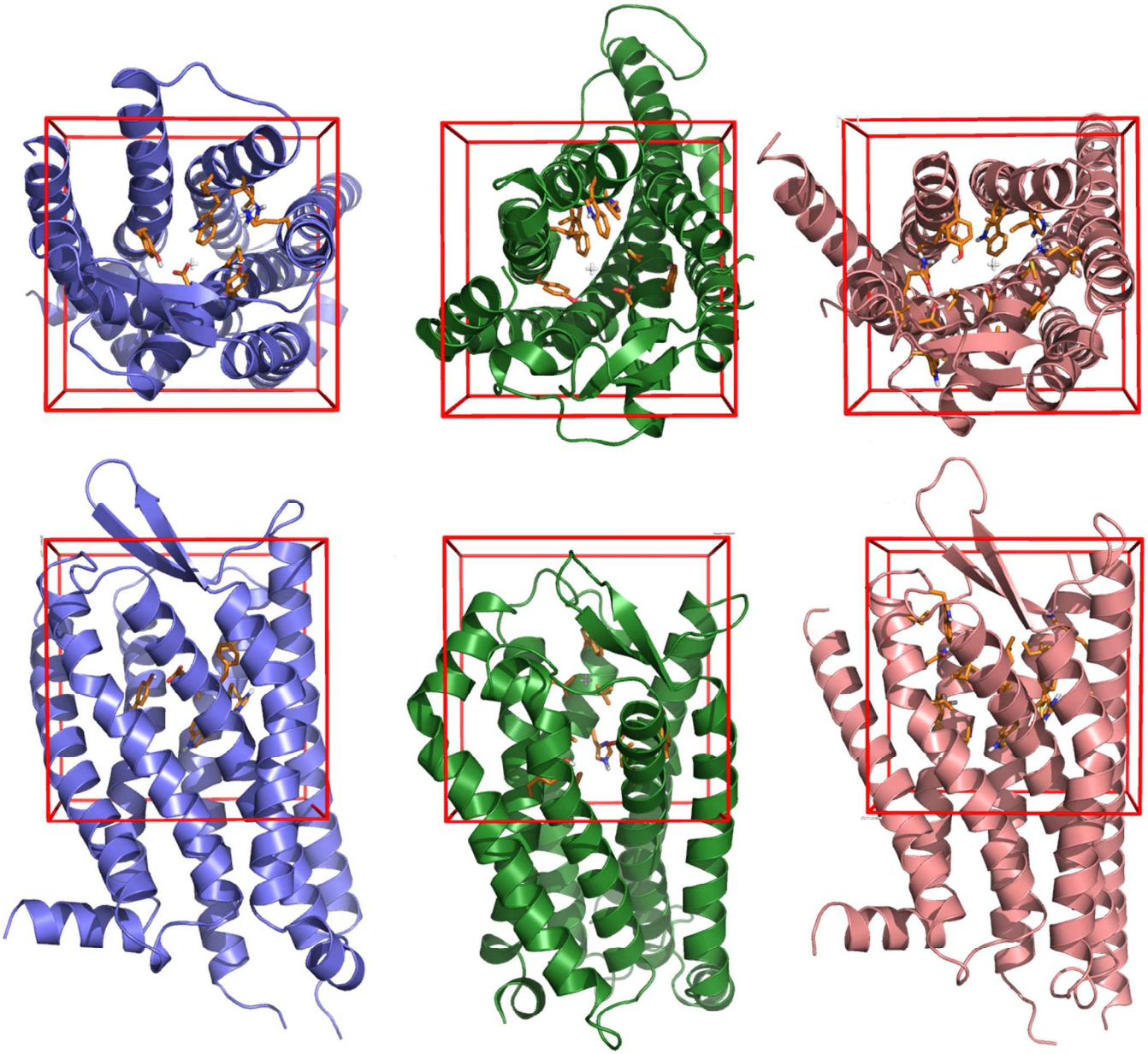


Figure S14. Docking grid volumes for mu (slate), delta (green) and kappa (salmon) in top (top) and side (bottom) view. Amino acid side chains constituting the binding pockets are shown in orange. N- and C-terminal tails are omitted for clarity.

Table S1: Binding affinity (K_i nM/pKi ± SEM) of **2** and **3** at select CNS receptors.

CMPD	5-HT1A	5-HT1B	5-HT1D	5-HT1E	5-HT2A	5-HT2B	5-HT5A	5-HT6	5-HT7	Alpha1A	Alpha1B	Alpha1D	Alpha2A
2													
3									3554 (5.45±0.07)				675 (6.2±0.1)

CMPD	Alpha2B	Alpha2C	BZP Rat Brain	D1	D2	D3	D4	DAT	M1	M2	M3	M4	M5	NET	SERT	Sigma 1	Sigma 2
2																	
3		992 (6±0.07)															

Binding affinities reported in Table S1 were conducted by the National Institute of Mental Health Psychoactive Drug Screening Program (NIMH-PDSP).(1) Details of the methods and radioligands used for the binding assays are available on the NIMH PDSP website at https://pdspdb.unc.edu/pdspWeb/content/PDSP%20Protocols%20II%202013-03-28.pdf. Blanks represent binding affinity of K_i > 10 μM. While **2** showed no affinity (K_i > 10 μM) at any tested CNS receptor, **3** showed at least ~500 fold selectivity for its primary target MOR-1 against non-opioid receptors.

References

1. Besnard J*, et al.* (2012) Automated design of ligands to polypharmacological profiles. *Nature* 492(7428):215-220.